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The Evolution of the Thermal Niche Across Locally Adapted Populations of the Copepod *Tigriopus californicus*

Christopher S. Willett* and Christine Son

Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3280

Thermal performance is a key component of fitness particularly for ectotherms living in thermally variable environments. Local adaptation can occur within populations of a species that inhabit regions with divergent thermal conditions, but this adaptation may result in trade-offs in other measures of fitness. If these trade-offs affect other aspects of thermal performance, several different patterns are possible (Huey and Kingsolver 1993). One potential pattern from a trade-off is a shift in the thermal niche, meaning that an organism that can handle a new range of higher temperatures can no longer handle colder temperatures as well. A second type of pattern is a generalist/specialist trade-off whereby populations may have broader thermal niches but lower fitness at optimal temperatures [i.e. "a jack-of-all-trades is a master of none" (Huey and Hertz 1984)]. Another possibility is that increased investment associated with local thermal adaptation (i.e. high temperature tolerance) may result in trade-offs in non-thermally dependent traits (Angilletta et al. 2003). The nature and structure of these trade-offs could determine the degree to which organisms will be able to respond to a changing climate.

The copepod *Tigriopus californicus* (Baker, 1912) has become an important system in which to study the evolution of local adaptation to the thermal environment. Geographically distinct populations of this copepod occur in upper intertidal pools along the Pacific coast from central Baja Mexico to Alaska. These populations often show high degrees of genetic divergence from one another indicating that levels of gene flow between populations can be very limited over long periods of time (Burton 1997; Edmands 2001; Willett and Ladner 2009). There is also a clear latitudinal gradient in high temperature survival that is suggestive of local thermal adaptation for this species (Willett 2010; Kelly et al. 2012; Leong et al. 2018). This latitudinal gradient for high temperature tolerance has been seen for nauplii and copepodids as well as adults (Tangwancharoen and Burton 2014).

Local thermal adaptation in *T. californicus* is also suggested by studies of fitness components and competitive fitness under non-extreme temperatures. Hong and Shurin (2015) examined 15 populations of *T. californicus* from Vancouver Island, BC, Canada, to southern California (CA) for a set of life history traits that contribute to fitness under four different temperature conditions (from 15°C to 30°C). They estimated the net fitness effect of these traits by calculating an intrinsic population growth rate (r) and found a consistent shift in the thermal niche from south to north and also higher r in the northern populations. Willett (2010) also found that for comparisons across a set of moderate temperatures there was a flip in competitive fitness between pairs of southern and central CA *T. californicus* populations. Central CA populations outcompeted southern populations at 16°C while the opposite pattern was observed in a fluctuating environment with an average temperature of 24°C (a 20°C to 28°C daily cycle). Combined these results suggest that

* Corresponding author: willett4@email.unc.edu

Table 1. Sample sizes of thermal stress assays for populations of *Tigriopus californicus*. Numbers indicate replicates with each replicate having 10 males and 10 females. Populations are arranged from the most southern (SRQ) to the most northern (FHL).

Population	37°	38°	39°	-2°
SRQ	5	10	8	23
BR	13	14	4	31
SD	8	8	-	16
CAT	7	10	4	21
AB	13	22	-	35
SC	9	8	-	17
BB	12	8	-	20
FHL	8	4	-	12

northern populations may achieve higher net fitness at intermediate temperatures but have lowered survival at high temperatures.

Differences in cold tolerance have also been observed across *T. californicus* populations for several different measures of function/survival at low temperatures (Wallace et al. 2014). *T. californicus* showed surprisingly high levels of cold tolerance with recovery from exposure to -2°C for all tested populations and recovery from freezing in the most northern populations. One central CA *T. californicus* population showed an unexpectedly low level of tolerance more comparable to southern CA populations than populations further north (in Washington State and British Columbia, Canada). When compared to results from high and moderate temperatures for other central CA populations this finding could suggest that this population has a narrowed thermal niche and could be more of a thermal specialist. However, the populations used by Wallace et al. (2014) were not the same as those used in other studies of this species, so we do not know both high and low temperature tolerance for these same populations that would enable us to get a full picture of the width of the thermal niche and how it changes across populations. In this study we explore both high and low temperature tolerance for a set of eight populations of *T. californicus* to gain a better understanding of the evolution of the thermal tolerance breadth and gain insights into the thermal niche width for this species.

The eight populations used in this study were selected for temperature assays because they span a wide portion of the range of this species and have recently been targeted for a comparative genomics study (Barreto et al. in prep.). These populations stretch from San Roque in Baja California Sur, Mexico (SRQ, 27°11′12″N, 114°23′52″W) to Friday Harbor in WA, USA ((FHL, 48°32′47″N, 123°0′35″W). The other populations from south to north were the California, USA populations of Bird Rock (BR, 32°48′54″N, 117°16′23″W), San Diego (SD: 32°44′44″N, 117°15′18″W), Catalina Island (CAT, 33°26.8′N, 118°28.6′W), Abalone Cove (AB, 33°44′16″N, 118°22′31″W), Santa Cruz (SC, 36°56′58″N, 122°02′49″W), and Bodega Bay (BB, 38°19′4″N, 123°4′23″W). Copepods were maintained in the laboratory at 20°C with a 12 hr:12 hr Light:Dark cycle for at least 1 yr before conducting the high temperature assays and 2 yr before conducting the cold tolerance assays.

Acute, high temperature stress tolerance assays were done by measuring survival 3 d after a 1 hr exposure to the stressful temperatures of 37°C, 38°C, or 39°C as described in Willett (2010). Sets of 10 males and 10 females were done for each population and the number of replicates for each temperature treatment is shown in Table 1. Not all populations were

tested at 39°C if high levels of mortality were observed for that population at 38°C. Survival of copepods under heat stress was modeled as binomial using a generalized linear model (function glm in R version 3.3.0; R Core Team 2016).

For the chill coma recovery assays, a modified version of the assay from Wallace et al. (2014) was used. Ten male and ten female copepods from a target population were placed in 10 mL of instant ocean seawater in a 50 mL centrifuge tube. Tubes were then placed in a chilled water bath at -2°C (containing a 50% ethylene gycol mixture) for 20 min. With this temperature exposure all copepods exhibited a chill coma phenotype wherein the copepods fell to the bottom and were immobile. Tubes were removed from the water bath and copepods were transferred to petri dishes where the seawater was allowed to return to room temperature while monitoring the recovery of copepods to an active state. Dishes were checked at roughly 2-min intervals to determine the number of copepods that had recovered and were swimming. Very limited mortality was observed for this cold stress exposure and copepods had largely all recovered by the time the plates had reached 14°C about 15 min after transferring them to the petri dishes. The time for recovery of 50% of copepods was used as our measure of chill coma recovery and analyzed as a generalized linear model in R.

The -2°C temperature was chosen to enable comparison to previous results (Wallace et al. 2014) but it is likely to be more environmentally realistic for the northern populations than the southern populations. Temperature data from nearby weather stations suggested that central CA locations may experience a small number of days below freezing while locations to the north in WA, and British Columbia, Canada experience more than 38 freezing days per year (Wallace et al. 2014) and southern CA locations experiencing no days below freezing. We looked at data from different weather stations near the populations used in this study and found a similar pattern. Over the last 12 yr the most northern FHL population had an average extreme low of -7.7°C, while the central CA locations SC and BB had values of -2°C and 0.5°C respectively, while locations near the southern CA populations of CAT and SD had average extreme lows of 4.7°C and 3.6°C respectively (data from NOAA at www.ncdc.noaa.gov/cdo-web/). The connection between pool temperature and nearby air temperatures is not direct and can be complicated by pool volume, substrate color, tidal timing, and other pool-specific environment factors but measured pool temperatures are more variable than nearby surface ocean temperatures (Kelly et al. 2012; Leong et al. 2018).

Copepods exposed to an acute, one-hour heat stress showed a general pattern of higher tolerance at lower latitudes (Fig. 1). The best fit model to these data includes the factors latitude, sex, and heat shock temperature. All main effects are significant (latitude $P = 0.001$, sex (males) $P = 0.01$, temperature $P<0.001$). Males have significantly lower acute stress tolerance than females, consistent with previous studies (Willett 2010). The southernmost population (SRQ) from central Baja California, Mexico showed much higher tolerance with some copepods surviving the 39°C exposure similar to previous results for this and nearby populations (Kelly et al. 2012; Pereira et al. 2017). There are some examples of regional variation that contrast with the general latitudinal pattern. In this dataset, the SD population has lower thermal tolerance than neighboring populations, a trend that has also been observed in previous studies for acute, high temperature assays (Willett 2010; Pereira et al. 2014). It is possible these local deviations from the latitudinal pattern could reflect finer scale differences in thermal adaptation.

Chill coma recovery time also followed a latitudinal gradient in which northern populations showed faster recovery than the southern populations, as would be expected

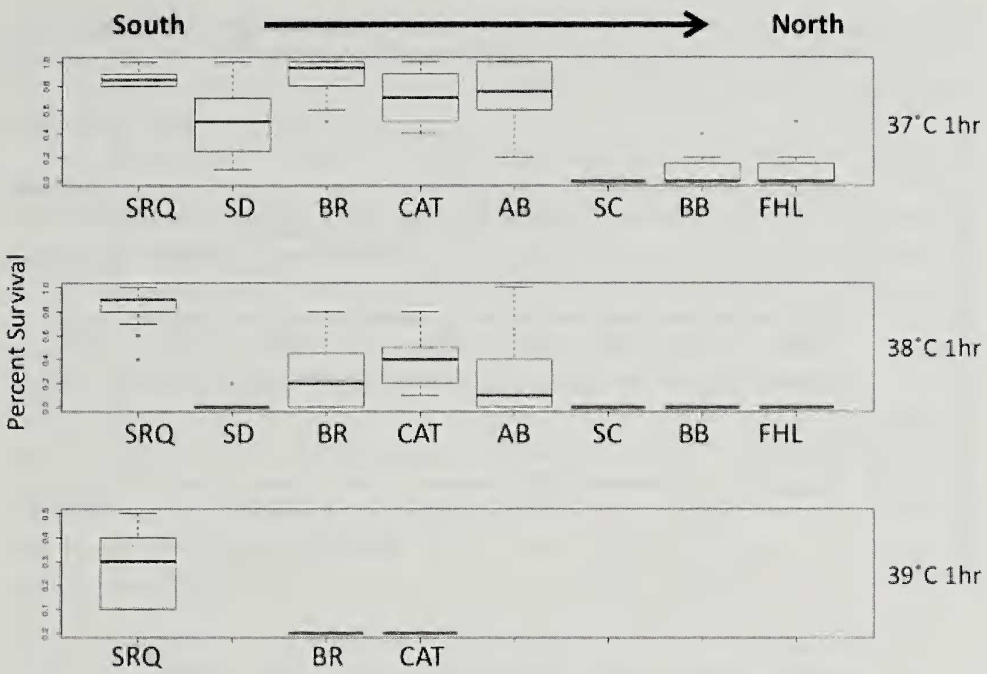

Fig. 1. High temperature acute stress assays for populations of *Tigriopus californicus*. Copepods were tested at three different temperatures (37°C top, 38°C middle, and 39°C bottom panel) for 1 hr and survival was measured 3 d after heat shock. Populations are arranged from south (on the left) to north (on the right). Plots show combined data for males and females. Box plots for each population/temperature combination depict median (bold line), first and third quartiles (box), largest non-outlier values (whiskers), and outliers (dots).

with local adaptation (Fig. 2). Both latitude and sex are significant factors in analyses of these data ($P<0.001$ for both) with males showing less thermal tolerance at colder temperatures (i.e. longer recovery times). Two populations of interest in comparison to the Wallace et al. (2014) results are the SC and BB populations from central CA. They showed an intermediate level of tolerance that falls between the recovery times of the more northern and southern populations. In the Wallace et al. study, the central CA population of Hopkins Marine Station (just south of SC) showed a cold temperature tolerance much more similar to a southern CA population proximate to the SD population included in our study. It is unclear why this Hopkins population showed this lower cold tolerance in the previous study and whether it also has correspondingly higher high temperature survival. In contrast, the results from our study suggest an intermediate both cold and hot temperature tolerance for the central CA populations with both increased cold tolerance and decreased heat tolerance in comparison to more southern populations but slightly decreased cold tolerance in comparison to the FHL population to the north.

Using a combination of measures of high and low thermal performance from the same populations, we can make stronger inferences about changes in the width of the thermal tolerance range among populations and how performance at extreme temperatures relates to performance at temperatures closer to optimal temperatures. Overall the results of our study are most consistent with a niche shift with an increase in high temperature tolerance and a decrease in cold temperature tolerance at lower latitudes and the opposite pattern

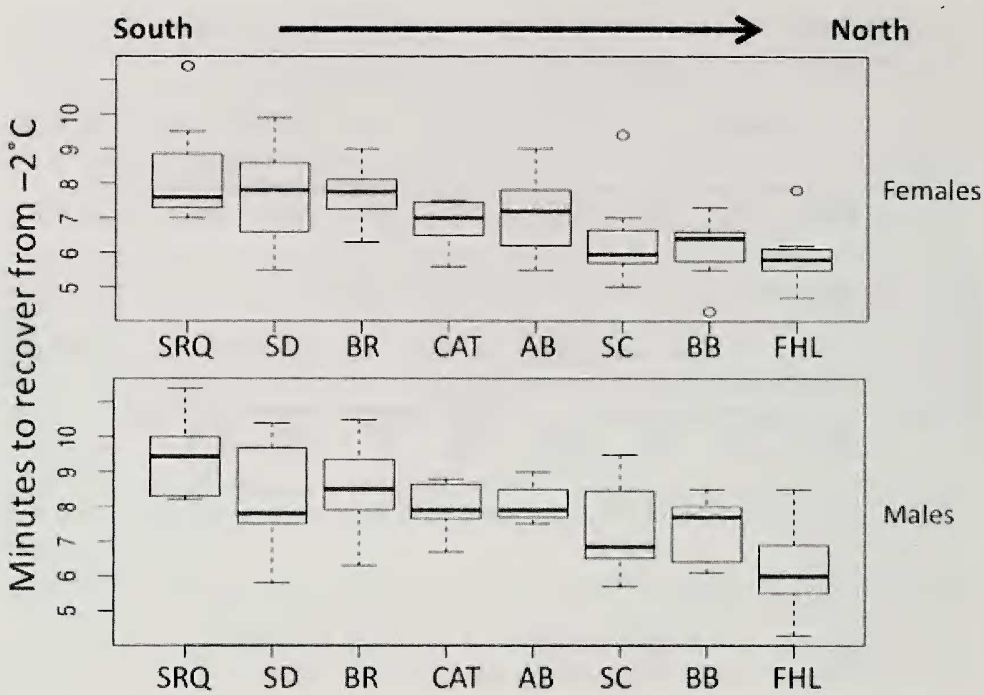

Fig. 2. Chill coma recovery for populations of *Tigriopus californicus*. The number of minutes for half of the copepods within an assay to recover from a -2°C exposure of 20 min is shown. Populations are arranged from south (on the left) to north (on the right). Results from females are shown on the top and males are on the bottom. Boxplots are as described in Fig. 1.

at higher latitudes. A niche shift was also suggested by the results of Hong and Shurin (2015) for testing over a range of moderate temperatures. If the central CA populations had shown narrower tolerance ranges this could have suggested that these populations were behaving more as thermal specialists (particularly when associated with the higher competitive fitness these populations show at intermediate temperatures in comparison to southern CA population; Willett 2010).

Combined with the results from previous studies, there is some evidence for not only a niche shift but also an increase in combined measures of fitness for more northern populations (Willett 2010; Hong and Shurin 2015). Hong and Shurin (2015) found a pattern of a niche shift towards lower temperatures with increasing latitude and also increasing composite fitness for the northern populations (as measured by the population growth rate r). As mentioned above, Willett (2010) found higher competitive fitness for central CA populations in competitive fitness assays. The increase in fitness with latitude is consistent with these populations showing a pattern of counter-gradient variation, perhaps due to stronger selection for shorter development due to a shorter growing season at more northern latitudes (Yamahira et al. 2007; Gardiner and Munday 2010; Hong and Shurin 2015). Faster development for northern populations has also been described by Edmands and Harrison (2003). Although niche width does not appear to decrease with increasing fitness for northern populations (which would have suggested a generalist/specialist trade-off), there may be trade-offs with survival and fecundity associated with the overall higher estimates of population growth rate for these higher latitude populations (Hong and Shurin

2015). Other studies of trade-offs in *T. californicus* have found no evidence for fitness trade-offs associated with laboratory selection for high temperature tolerance (Kelly et al. 2013) but potential trade-offs when there are joint salinity and high temperature stresses (Kelly et al. 2016; Leong et al. 2018).

Given the low levels of gene flow among populations of *T. californicus*, if the environment becomes less suitable due to future climate change and conditions begin to exceed their thermal limits, individual populations continued survival would require evolutionary adaptation as it would be less likely that immigrants from more tolerant populations will arrive to rescue populations by introducing more tolerant alleles. Kelly et al. (2012) suggest that there is limited potential for selection to improve heat tolerance in *T. californicus* over relatively short time periods for any single population. The results from the current study suggest that all of the populations show roughly similar thermal niche widths but horizontal shifts from south to north and this is likely to mean that sensitivity to future change will depend on specific scenarios of environmental change for each location. If higher temperature stresses become more common, copepod fitness could be negatively impacted as conditions in the field have been found to approach the lethal temperatures measured in these high temperature assays (Kelly at el. 2012; Leong et al. 2018). Further complications in predicting future responses of *T. californicus* populations to changing temperatures could also stem from potential differences in the physiological mechanism underlying response to thermal variation across populations. Even populations with similar phenotypic responses to thermal stress can show dramatically different patterns of gene expression (Lima and Willett 2017) suggesting differences in how these populations respond at the physiological level. Therefore, these populations may respond differently to selection and have different abilities to adapt to changing environments.

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